Relative Effects of Ultraviolet and Visible Light on the Activities of Corn Earworm and Beet Armyworm (Lepidoptera: Noctuidae) Nucleopolyhedroviruses

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ABSTRACT Five different combinations of fluorescent tubes (UV-B/UV-B, UV-B/UV-A, UV-A/UV-A, UV-B/White, White/White) were used to determine relative effects of UV and visible light on the nucleopolyhedroviruses (NPV) of *Helicoverpa zea* and *Spodoptera exigua.* For both viruses, the greatest inactivation occurred with exposure to UV-B radiation. Both virus concentration and radiation exposure time influenced the rate and degree of inactivation. In the case of the UV-A/UV-A and White/White combinations inactivation occurred only with the longest exposure (24 h) and the lowest virus concentration (0.747 PIB/mm^2). The NPV from *H. zea* was found to be more sensitive to UV radiation than the NPV from *S. exigua.*

KEY WORDS *Helicoverpa zea*, *Spodoptera exigua*, ultraviolet light, visible light

ONE OF THE MOST important factors limiting the effectiveness of insect pathogenic viruses as microbial control agents is sensitivity to solar radiation (Jaques 1968, Bullock et al. 1970, Jones and McKinley 1986). In many cases, field-applied pathogens lose at least 50% of their original activity within several days (Ignoffo et al. 1977), and in many cases within 24 h (Broome et al. 1974). Previous studies (David 1969, Bullock et al. 1970, Harms et al. 1986) have shown that viral inactivation is more efficicient at wavelengths of 280–290 nm than at 320–330 nm, but that inactivation could occur from 290 to 380 nm. Jones and McKinley (1986) demonstrated that >90% inactivation of *Spodoptera littoralis* nucleopolyhedrovirus (SlMNPV) occurred within 4 h and that >99% inactivation occurred within 8 h, under natural conditions. They concluded that almost all of the inactivation was due to UV-B (i.e., 305–320 nm). Morris (1971) exposed the western hemlock looper, *Lambdina fiscellaria lugubrosa* (Hulst), NPV to UV-A (366 nm) radiation for periods of up to 100 h and obtained some inactivation. As the exposure period increased, however, so did the mean time to death and the LT_{50}. In the case of the entomopathogenic bacterium *Bacillus thuringiensis* Berliner, exposure to UV-A (365 nm) and visible (405 nm) radiation was shown to cause single strand breaks and DNA-protein cross links (Harms et al. 1986).

Although exposure to the UV portion of the solar spectrum, especially 280–320 nm (UV-B) is thought to be primarily responsible for inactivation (David 1969, Jaques 1977, Jones and McKinley 1986), some evidence exists that UV-A may also play a role in viral inactivation (Witt and Stairs 1975, Shapiro and Robertson 1990).

This research was not designed to determine precisely those wavelengths responsible for inactivation but should provide data on the relative effects of UV and visible light of NPVs of the corn earworm, *Helicoverpa zea* (Boddie), and the beet armyworm, *Spodoptera exigua* (Hübner), using inexpensive UV-white light tubes.

Materials and Methods

Insects and Virus Inocula. The colonized strains of the corn earworm, *H. zea*, and the beet armyworm, *S. exigua*, established and maintained by USDA-ARS, Tifton, GA, were used. Larvae were reared on a wheat germ diet developed for gypsy moth (Bell et al. 1981), but a carrageenan Gelcarin DG 611 (FMC, Rockland, ME) was substituted for agar. Nucleopolyhedroviruses from both the corn earworm (HzSNPV) and the beet armyworm (SeMNPV) were obtained from Certis U.S.A. (Columbia, MD) and were passed in the homologous hosts before being assayed against *H. zea* and *S. exigua*, respectively.

Bioassays. Viral inclusion bodies from both nucleopolyhedroviruses were extracted from virus-killed larvae (Shapiro et al. 1981). The insects were homogenized (e.g., each gram of insect tissue was blended in 9 gm distilled water) and filtered through coarse cheesecloth, and the filtrate was collected (=stock virus suspension). One milliliter of the stock suspension was diluted in 9 ml distilled water. A sample was removed by Pasteur pipette and the concentration of this suspension (1:10) was determined using a double-

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line hemacytometer with improved Neubauer ruling and phase microscopy (430× magnification). Dilutions were made from the stock suspension to produce concentrations ranging from 10^2 to 10^7 polyhedral inclusion bodies (PIB)/ml and PIB suspensions were applied to the surface of the diet (0.1 ml/30-ml cup; 1,338 mm^2 = surface area) at final concentrations ranging from 10 to 1 million PIB/30 ml cup (=0.0074–744.4 PIB/mm^2).

Second instars (4 d old) were placed individually in each container and were reared for 14 d at 29°C, 50% RH, and a photoperiod of 12:12 (L:D) h. Tests were repeated six times with 10 larvae per virus dilution per replicate and 10 control larvae per replicate. Mortality was assessed initially at day 3 and every 2–3 d thereafter until day 14, when the tests were terminated.

Statistical Methods. Concentration-mortality regressions were estimated by probit analysis (LeOra Software 1987) to monitor the biological activities of HzSNPV and SeMNPV. Failure of 95% CL to overlap was used as the criterion for significant differences at LC values.

Radiation Source. Radiation was provided by two fluorescent tubes (15 W, 382 mm, Fotodyne, New Berlin, WI), which were mounted in parallel within an aluminum reflector (Fisher, Pittsburgh, PA) 203.2 mm above the test dishes. HzSNPV and SeMNPV suspensions were exposed for periods ranging from 5 min to 24 h. The different light combinations used were: UV-B/UV-A (standard), UV-B/UV/B, UV-A/UV-A, UV-B/visible light, UV-A/visible light, visible light/visible light (=White/White).

Determination of Radiation Intensity. The irradiance of various paired sets of tubes, whose energy outpoint was centered at 312 nm (UV-B), 366 nm (UV-A), and 550–580 nm (white light) was measured with a spectroradiometer system (International Light, Newburyport, MA). Input optics consisted of a 15° quartz assembly (model SLW), which delivered light to the single-grating monochromator with selectable filters and optimized for UV at 240 nm. The monochromator was directly coupled to a high gain photomultiplier (IL 782A), which fed current to the radiometer (IL 1700) used to convert measurement to watts/cm^2. All irradiance measurements correspond to a distance of 204 mm between the light source and the input optics.

Exposure of NPVs to UV and Visible Light. For these tests, 4 ml of virus suspensions ranging from 10^4 to 10^7 PIB/ml were pipetted into 60 by 15-mm glass petri dishes (Fisher, Pittsburgh, PA) and were exposed for periods ranging from 5 min to 24 h. After the exposure periods, the remaining volumes were determined and distilled water was added to each dish to replace water lost by evaporation. Lids were then placed on all dishes, and dishes were stored at 4°C until usage.

NPV Bioassays. A bioassay was then conducted with virus concentrations ranging from 10^1 (=0.0074 PIB/mm^2) to 10^6 PIBs/cup (=744.4 PIB/mm^2) was also conducted to compare biological activities before and after irradiation. For testing, 0.1 ml of virus suspension (light-exposed and nonexposed) was pipetted onto the diet surface of each 30-ml cup. Second instars (4 d old) were placed individually in each container and reared for 14 d at 29°C, 50% RH, and a photoperiod of 12:12 (L:D) h. Tests were repeated six times with 10 larvae per virus dilution per replicate and 10 control larvae (untreated) per replicate. Mortality was assessed initially at day 3 and every 2–3 d thereafter until day 14, when the tests were terminated.

Inactivation Data. For these tests, the biological activities of unexposed NPV at concentrations ranging from 10^3 PIB/cup (=0.774 PIB/mm^2) to 10^6 PIB/cup (=744.4 PIB/mm^2) were used as standards for comparison with NPV (10^3 to 10^6 PIB/cup) that was exposed to radiation for periods ranging from 5 min to 1,440 min. The percentage of original activity remaining was based upon virus-caused mortality before and after irradiation at the same virus concentration (e.g., 10^4 PIB/cup) for each exposure period for each treatment (Ignoffo and Batzer 1971).

Results

The initial step for UV inactivation studies was to obtain concentration-mortality Data for unexposed NPV, which provided bas line data for subsequent inactivation studies. Differences in the amounts of virus required to produce an LC_{10}, LC_{30}, LC_{50}, LC_{70}, and LC_{90} both HzSNPV and SeMNPV were nonsignificant ($P = > 0.05$) at every point in the concentration-mortality curve. For example, the LC_{50} value of 562 PIB/cup (95% CL = 468–669) and LC_{90} of 4,332 PIB/cup (95% CL = 3,255–6,090) for HzSNPV were statistically similar ($P = > 0.05$) to the LC_{50} value of 375 PIB/cup (95% CL = 335–937) and LC_{90} value of 4,375 PIB/cup (95% CL = 2,194-15,481) for SeMNPV. For previous UV inactivation studies, a concentration-mortality curve was obtained and a single concentration producing 90–95% mortality was used (Shapiro et al. 1992). For this study, however, four concentrations were used (e.g., 0.747, 7.473, 74.738, and 747.384 PIB/mm^2 or 10^3, 10^4, 10^5, and 106 PIB/cup), which represented LC values ranging from $\approx LC_{50}$ to $> LC_{99}$.

Measured outputs from the five different combinations of fluorescent tubes are Shown in Table 1. The combination of two UV-B tubes produced the greatest energy output (=7064.2 × 10^{-8} W/cm^2), followed by the UV-B/UV-A combination (=67.6% that of UV-B/UV-B), the White/White combination (=56.5% that of UV-B/UV-B), UV-A/UV-A (=5.5% that of UV-B/UV-B), and the UV-B/White combination (=48.0% that of UV-B/UV-B) (Table 1). The energy profiles of the different combinations were also determined and each combination was different. For example, UV-B/UV-B energy output consists of a combination of UV-B (55%), UV-A (41%), and visible radiation, whereas the UV-B/White combination consisted of ≈ equal amounts of UV-B (32%), UV-A (30%), and visible radiation (38%). In the UV-A/UV-A combination, 88% of the total energy was emitted as UV-A radiation (88%), whereas 95% of total energy emitted in the

Table 1. Energy outputs of UV-B, UV-A, and white light fluorescent tubes used in different combinations for inactivation studies (measured with a spectroradiometer as $\times 10^{-8}$ W/cm^2)

Energy output at different wavelengths	Combinations of tubes used for inactivation studies				
	UV-B/UV-B	UV-B/UV-A	UV-A/UV-A	UV-B/White	White/White
280–320 nm (UV-B)	3,911.8	1,403.7	105.4	1,091.1	30.0
320–400 nm (UV-A)	2,899.0	2,952.5	3187.7	1,005.1	193.0
400–800 nm (White)	252.5	421.9	342.9	1,294.3	4,001.1
Total energy	7,064.2	4,778.1	3,636.0	3,390.5	4,224.1
% UV-B/Total	55.4	29.4	2.9	32.2	0.7
% UV-A/Total	41.1	61.8	87.7	29.6	4.6
% White/Total	3.5	8.8	9.4	38.2	94.7

White/White combination consisted of visible radiation.

The UV-B/UV-B, UV-B/UV-A, and UV-B/white light combination tubes were effective in inactivating both viruses and the effects were dependent upon both the length of UV exposure and the virus concentration (Table 2). For example, at a concentration of 0.747 PIB/mm^2 HzSNPV-caused mortality was reduced from 66.7% (0 UV) to 0% after a 5-min exposure to the UV-B/UV-B tubes. At an LC95 (=7.473 PIB/mm^2), mortality was reduced from 96.7% (0 UV) to 6.7% after a 5-min exposure (=6.9% OAR) and 100% inactivation (=0 mortality) occurred between 10 min (=1.3% mortality; = 1.3% OAR) and 15 min. As the virus concentration was increased to 73.738 PIB/mm^2; >LC_{99}), total inactivation occurred between 15 min (=3.3% mortality) and 30 min (=0 mortality). At the highest concentration used (747.384 PIB/mm^2, >LC_{99}) 18.3% mortality still occurred after 30 min (Table 2).

The UV-B/UV-A and UV-B/white light tubes also reduced activity of HzSNPV, but these combinations were not as effective as the UV-B/UV-B combination. For example, at the highest virus concentration (=747.384 PIB/mm^2; >LC_{99}) total inactivation occurred between 15 and 30 min after exposure to UV-B/UV-B tubes, but activity still occurred after a 120-min exposure to the UV-B/White light combination

Table 2. Effects of ultraviolet and visible light combinations on the biological activities of the corn earworm NPV

Virus	Radiation system	Virus concn (PIB/mm^2)	% mortality after irradiation[a,b]							
			Exposure time, min							
			0	5	10	15	30	60	120	240
HzSNPV	UV-B/UV-B	0.747	66.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		7.473	96.7	6.7	1.3	0.0	0.0	0.0	0.0	0.0
		74.738	>99.0	>99.0	>99.0	10.0	5.0	3.3	0.0	0.0
		747.384	>99.0	95.0	70.0	50.0	18.3	0.0	0.0	0.0
	UV-B/UV-A	0.747	57.3	8.3	0.0	0.0	0.0	0.0	0.0	0.0
		7.473	97.3	21.7	5.0	0.0	0.0	0.0	0.0	0.0
		74.738	>99.0	50.0	31.7	20.0	5.0	0.0	0.0	0.0
		747.384	>99.0	>99.0	>99.0	90.9	60.0	45.0	25.0	8.3
	UV-A/UV-A	0.747	56.7	—	—	—	—	56.7	58.3	55.0[c]
		7.473	80.0	—	—	—	—	71.7	76.7	80.0[d]
		74.738	93.3	—	—	—	—	98.3	98.3	100.0[e]
		747.384	>99.0	—	—	—	—	>99.0	>99.0	>99.0[f]
	UV-B/White	0.747	53.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		7.473	81.7	21.7	3.3	0.0	0.0	0.0	0.0	0.0
		74.738	98.3	45.0	10.0	0.0	0.0	0.0	0.0	0.0
		747.384	>99.0	95.0	83.3	61.7	38.3	18.3	11.7	0.0
	White/White	0.747	63.3	—	—	—	—	65.0	68.3	63.3[g]
		7.473	95.0	—	—	—	—	>99.0	93.3	95.0[h]
		74.738	>99.0	—	—	—	—	>99.0	98.3	>99.0[i]
		747.384	>99.0	—	—	—	—	>99.0	>99.0	>99.0[j]

[a] Six replicates; 10 larvae per virus concentration per replicate; 10 untreated larvae per replicate.

[b] To determine percent original activity remaining (percent OAR) divide the mortality at a given virus concentration and at any given exposure period to irradiation by the mortality at the same concentration without any irradiation (=0 exposure). For example, the percent OAR for HzSNPV at a concentration of 0.747 PIB/mm^2 for a 5-min UV-B/UV-B exposure is determined by dividing the percent mortality of UV-exposed NPV (= 0.0% kill) by the percent mortality of unexposed NPV (= 66.7%) $\times$ 100 = 0.0% OAR.

[c] Percent mortality at 6 h exposure = 48.3; percent mortality at 24 h exposure = 13.3.

[d] Percent mortality at 6 h exposure = 78.3; percent mortality at 24 h exposure = 48.3.

[e] Percent mortality at 6 h exposure = 96.7; percent mortality at 24 h exposure = 78.3.

[f] Percent mortality at 6 h exposure = >99%; percent mortality at 24 h exposure = 100.0.

[g] Percent at 6 h exposure = 53.3; percent mortality at 24 h exposure = 8.3.

[h] Percent mortality at 6 h exposure = 80.0; percent mortality at 24 h exposure = 36.0.

[i] Percent mortality at 6 h exposure = >99%; percent mortality at 24 h exposure = 83.3.

[j] Percent mortality at 6 h exposure = >99%; percent mortality at 24 h exposure = >99%.

Table 3. Effects of ultraviolet and visible light combinations on the biological activities of the beet armyworm NPV

Virus	Radiation system	Virus concn (PIB/mm^2)	Exposure time, min[a,b]							
			0	5	10	15	30	60	120	240
SeMNPV	UV-B/UV-B	0.747	55.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		7.473	93.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		74.738	>99.0	40.0	21.7	13.3	5.0	1.7	0.0	0.0
		747.384	>99.0	93.3	80.0	71.7	43.3	21.7	16.7	6.7
	UV-B/UV-A	0.747	60.0	40.0	15.0	10.0	5.0	0.0	0.0	0.0
		7.473	92.2	73.3	51.7	18.9	1.7	0.0	0.0	0.0
		74.738	98.9	65.0	50.0	12.2	1.1	0.0	0.0	0.0
		747.384	>99.0	>99.0	>99.0	96.7	85.0	65.0	38.3	6.7
	UV-A/UV-A	0.747	66.7	—	—	—	—	58.3	63.3	50.0[c]
		7.473	91.7	—	—	—	—	88.3	86.7	76.7[d]
		74.738	98.3	—	—	—	—	>99.0	98.3	98.3[e]
		747.384	>99.0	—	—	—	—	>99.0	>99.0	>99.0[f]
	UV-B/White	0.747	56.7	50.0	3.3	0.0	0.0	0.0	0.0	0.0
		7.473	81.7	70.0	40.0	8.3	0.0	0.0	0.0	0.0
		74.738	98.3	91.7	58.3	30.0	16.7	3.3	0.0	0.0
		747.384	>99.0	>99.0	>99.0	93.3	76.7	31.7	18.3	3.3
	White/White	0.747	63.3	—	—	—	—	65.0	68.3	63.3[g]
		7.473	95.0	—	—	—	—	>99.0	93.3	95.0[h]
		74.738	>99.0	—	—	—	—	>99.0	98.3	>99.0[i]
		747.384	>99.0	—	—	—	—	>99.0	>99.0	>99.0[j]

[a] Six replicates; 10 larvae per virus concentration per replicate; 10 untreated larvae per replicate.

[b] To determine percent original activity remaining (% OAR) divide the mortality at a given virus concentration and at any exposure period post-irradiation by the mortality at the same concentration without irradiation (= 0 exposure). For example, the percent OAR for SeMNPV at a concentration of .747 PIB/mm^2 for a 5-min UV-B/UV-B exposure is determined by dividing the percent mortality of UV-exposed NPV (= 0.0% kill) by the percent mortality of unexposed NPV (= 55.0%) × 100 = 0.0% OAR.

[c] Percent mortality at 6 h exposure = 48.3; percent mortality at 24 h exposure = 13.3

[d] Percent mortality at 6 h exposure = 78.3; percent mortality at 24 h exposure = 48.3

[e] Percent mortality at 6 h exposure = 96.7; percent mortality at 24 h exposure = 78.3.

[f] Percent mortality at 6 h exposure = > 99%; percent mortality at 24 h = >99%.

[g] Percent mortality at 6 h exposure = 53.3; percent mortality at 24 h exposure = 8.3.

[h] Percent mortality at 6 h exposure = 80.0; percent mortality at 24 h exposure = 36.0.

[i] Percent mortality at 6 h exposure = >99.0; percent mortality at 24 h exposure = 83.3.

[j] Percent mortality at 6 h exposure = >99.0; percent mortality at 24 h exposure = >99.0.

and a 240-min exposure to the UV-B/UV-A combination (Table 2). The UV-A/UV-A and White/White light combinations had no apparent detrimental effects upon HzSNPV activity after a 240-min exposure but some inactivation occurred after a 24-h exposure (Table 2).

In the case of SeMNPV, a similar inactivation profile was obtained (Table 3). As the virus concentration increased, the time required to cause 100% inactivation also increased after exposure to UV-B combinations. In the case of UV-A/UV-A and White/White treatments, viral inactivation occurred only at the two lowest virus concentrations after a 240-min exposure. As the exposure period was increased to 24 h, inactivation occurred at all virus concentrations but the highest (=747.384 PIB/mm^2) for both treatments. As a result of the 24-h treatments, only 23.2% and 13.1% original activity remained for the UV-A/UV-A and White/White treatments, respectively (Table 2).

The inactivation profile for SeMNPV (Table 3) was similar to that obtained for HzSNPV (Table 2). Thus, all UV-B combinations was detrimental to virus, whereas the UV-A/UV-A and White/White combinations were active only at the two lowest virus concentrations after a 360-min exposure and were not active for the highest virus concentration after a 24-h exposure (Table 3).

Discussion

The detrimental effects of solar radiation on insect viruses have been known for the past 50 yr (Watanabe 1951, David 1965, Bullock 1967), but a complete under-standing has not yet been reached (Ignoffo et al. 1977, Hunter-Fujita et al. 1998). In general, the research on UV-B has been much more extensive than that on UV-A, because of the greater energy output (David et al. 1968, Smirnoff 1972, Parrish et al. 1981) and greater biological activity of the former (Timans 1982, Sliney 1983, Lavker and Kaidbey 1997). It is well documented that UV-B radiation can inactivate insect viruses (David et al. 1968, Bullock et al. 1970) at a much faster rate than UV-A irradiation (Bullock et al. 1970, Timans 1982). It is assumed that UV-A plays a minor role in the inactivation process. However, UV-A is known to have detrimental effects on mammalian DNA by generation of reactive oxygen species (Runger et al. 1995, Ito and Kawanishi 1997) and direct damage to DNA by photooxidation of cytosine and guanine (Zhang et al. 1997, Kuluncsics et al. 1999).

During the past 30 yr, investigators have studied the relationship of UV-B, UV-A, and visible light to biological activity of entomopathogens. David et al. (1968), studying inactivation of a granulosis virus of *Pieris rapae*, assumed that sunlight inactivation occurred between 310 and 360 nm. In a subsequent

study, however, David (1969) exposed *Pieris rapae* to known wavelengths from 250 to 330 nm and found that inactivation occurred at all wavelengths. Although inactivation occurred throughout the UV-B spectrum, the amount of energy required to inactive virus at 320 nm was ≈ 160-times more than was required at 290 nm. Bullock et al. (1970) exposed HzSNPV to known wavelengths of UV-C (253.7 nm), UV-B (307.5 nm), and UV-A (365 nm) and to a broad band (visible light-near-infrared) for a 2-h period. Whereas inactivation was significant at UV-C and UV-B, nonsignificant loss in activity occurred at the UV-A and visible to near infra red treatments. Shapiro and Robertson (1990) tested 79 dyes as UV protectants for LdMNPV at an LC_{95}. A composite UV absorption profile of six effective dyes was compared with that from a representative composite sample of ineffective dyes. Both groups of dyes displayed similar absorbance patterns in the UV-B portion of the solar spectrum. In the UV-A portion, however, the total absorbance from 320–400 nm decreased among the ineffective dyes (i.e., by 16%), whereas the total absorbance of effective dyes increased substantially (e.g., by 200%) as the spectrum shifted from UV-B to UV-A. Morris (1985), working with the bacterium *Bacillus thuringiensis* Berliner, concluded that materials should be good UV absorbers at 330–400 nm, to be effective protectants. Whereas Pusztai et al. 1991 concluded that UV-B/UV-A (i.e., 300–380 nm) was primarily responsible for inactivation of *B. thuringiensis crystals*, Griego and Spence (1978) reported that mortality of *B.thuringiensis* spores was caused by irradiation at both UV and visible (400 nm) wavelengths. Harms et al. (1986) demonstrated that UV-A (365 nm) and visible (405 nm) radiation were detrimental to *B. thuringiensis* and caused single strand breaks and DNA-protein crosslinks.

The purpose of the current study was to determine the relative inactivation activities of UV-B, UV-A and visible light by themselves and in different combinations and to determine whether differences in sensitivity occurred between two NPVs (e.g., HzSNPV and SeMNPV), using a simple, inexpensive inactivation system. The purpose was neither to simulate natural sunlight, nor to determine precisely which wavelength or wavelengths was primarily responsible for inactivation of the viruses, using an inexpensive tube system. Future studies will use transmission filters to more precisely pinpoint those wavelengths most responsible for virus inactivation and to test specific radiation protectants.

Each combination of tubes not only produced radiant energy at different wavelengths but also produced different amounts of radiant energy. In terms of radiation produced (i.e., UV-B versus UV-A versus visible light), the most homogeneous combinations used were UV-A/UV-A (87.7% of total energy emitted as UV-A, 2.9% as UV-B, 9.4% as visible light) and White/White (94.7% of total energy emitted as visible light, 4.6% as UV-A, and 0.7% as UV-B). The least homogeneous systems were UV-B/UV-B (55.4% energy emitted as UV-B, 41.0% as UV-A, and 3.6% as visible light), UV-B/White (32.2% energy emitted as UV-B, 29.6% as UV-A, and 38.2% as visible light), and UV-B/UV-A (29.4% energy emitted as UV-B, 61.8% as UV-A, and 8.8% as visible light).

The UV-B/UV-B combination produced the greatest amount of total radiation, regardless of wavelength, (e.g., $7{,}064.2 \times 10^{-8}$ W/cm^2), followed by UV-B/UV-A (=4,778.1), White/White (4,224.1), UV-A/UV-A (3,636.0), and the UV-B/White ($3{,}390.5 \times 10^{-8}$ W/cm^2) combination. Since the most active combination (=UV/B-UV-B) also produced the most energy, was activity (=virus inactivation) totally dependent upon the total amount of energy? The UV-B/UV-B system ($=7{,}064.2 \times 10^{-8}$ W/cm^2) not only produced 1.5-fold and 1.9-fold more total radiation than the UV-B/UV-A and UV-B/White light systems, respectively, and produced 2.8-fold and 3.6-fold more UV-B radiation than the UV-B/UV-A and UV-B/White systems, respectively, but was the most active system (e.g., in terms of virus inactivation). The data confirm the activity of UV-B (Tables 1 and 2), but still raise some questions. Since the UV-B/White combination had greater activity than the UV-B/UV-A combination (Tables 2 and 3) but produced 22% less UV-B radiation and 29% less total radiation than the UV-B/UV-A combination (Table 1), what roles do UV-A and visible light play? Since the UV/B-White light system was almost as active as the UV-B/UV-B system but produced 72.2% less UV-B radiation and 52.0% less total radiation than the UV-B/UV-B system, what is the UV-B threshold level for virus inactivation?

In general, the activities of UV-A/UV-A and White/White were similar (Table 2) and two general conclusions can be made: (1) inactivation of virus was inversely proportional to virus concentration, and (2) inactivation of virus was directly proportional to radiation exposure time. At the lowest virus concentration (0.747 PIB/mm^2), virus inactivation did not occur until a 360-min exposure to either UV-A/UV- A or to White/White and increased during the next 18 h. As the virus concentration increased, a longer exposure period was required to inactivate virus at concentrations causing 90 to >99% mortality. At concentrations $>LC_{99}$ little if any inactivation occurred (Tables 2 and 3), which demonstrates the low activities of UV-A (320–400 nm) radiation and visible (400–800 nm) radiation. A possible cautionary note to this statement the fact that both UV-A/UV-A (87.7% UV-A, 9.4% white light) and White/White (4.6% UV-A, 94.7% white light) systems also emit small amounts of UV-B radiation (i.e., 2.9% for UV-A/UV-A, and 0.7% for White/White). Whether these low amounts of UV-B radiation in the UV-A/UV-A and White/White combinations are sufficient to cause virus inactivation will be the focus of further research. Woolons et al. (1999) determined that tanning lamps, which are predominantly UV-A emitters also emit UV-B radiation (i.e., 0.8% of total emission). UV-B radiation was found to be responsible, however, for producing 75% of the cyclobutane pyrimidine dimers and 50% of the oxidative damage to human skin DNA.

Despite the fact that the precise wavelengths responsible for virus inactivation were not determined, results obtained are consistent with those obtained on the relative activities of UV-B, UV-A, and visible radiation in various biological systems, including insect pathogens. UV-B had the greatest biological activity, irrespective of the systems used (Timans 1982, Harms et al. 1986, Andley et al. 1994). UV-A and visible light possessed biological activities (Cadet et al. 1997, Kielbassa et al. 1997) but virus inactivation occurred only at low virus levels (e.g., LC_{50}- LC_{95}) over prolonged exposure periods. Moreover, evidence also exists that UV-A, and possibly visible radiation, acts to enhance the biological activity of UV-B (Harms et al. 1986, Gange 1988). In the current study, the UV-B/white light system appeared to have greater activity than the UV-B/UV-A system but further research, using transmission filters, is needed to more precisely ascertain relationships among UV-B, UV-A, and visible light systems.

The biological activities of both viruses (0 radiation) were very similar and it was anticipated that they would be equally sensitive to UV radiation. When the data were examined, however, this premise was shown to be incorrect. At all concentrations tested, HzSNPV was more sensitive to the three UV-B systems than SeMNPV (Tables 2 and 3). These differences in relative sensitivity to UV radiation indicate that inherent differences exist among viruses. Gudauskas and Canerday (1968), using a UV-C (253.7 nm) radiation source, demonstrated that the *H. zea* NPV was more sensitive to radiation than the NPV from the cabbage looper, *Trichoplusia ni,* (Hübner). Moreover, inherent differences in biological activity and in UV-sensitivity within a given NPV geographical isolate (Abington, MA, isolate of LdMNPV) led to the selection of a more active biotype (Shapiro et al. 1992) and a more UV-tolerant biotype (Shapiro and Bell 1984). Different sensitivities to UV and visible light were also demonstrated among different isolates (=subspecies) of the bacterium *B. thuringiensis* (Harms et al. 1986).

For UV inactivation tests most investigators used virus concentrations that caused between 90–95% mortality and exposed virus to one or more exposure periods (Manjunath and Mathad 1981, Shapiro 1989, Ignoffo et al. 1991). The use of an LC_{90-95} leaves important questions unanswered: "How are laboratory data transferred to the field?" "Is the amount of virus used in the laboratory applicable in the field for control of insects?" These are not just academic questions, because the amount of virus applied is important for field performance (Chapman and Bell 1967, Webb et al. 1993). In the current study, virus concentrations exceeding LC_{99} were used to determine the influence of concentration upon inactivation and to use the data for subsequent small field tests. Payne (1982) stated that it was difficult to determine the inactivation of the virus, since ". . . the initial amount of virus deposited on the crop has caused 100% mortality of the test larvae in bioassays . . ." and recommended the use of $LC{\approx}_{50}$s (Ignoffo and Batzer 1971) to determine inactivation rates. The dangers of using an LC_{50} only are two-fold: first, the inactivation rates are different at the LC_{50} and greater LC values (Tables 2 and 3), and second, how does this mortality level relate to the amount of virus to be applied in the field for practical control? Using the UV-B/UV-A system as an example, for HzSNPV ($\approx LC_{50}$) 84.5% inactivation occurred after a 5-min UV exposure, and no activity remained after an additional 5-min exposure. For SeMNPV ($\approx LC_{50}$) inactivation was a slower process (i.e., 33.3% loss at 5 min, 75.0% loss after 10 min, 83.3% loss after 15 min, 91.2% loss after 30 min, 100% loss after 60 min).

We can also look at the data in another way and approximate the amount of active virus remaining after UV irradiation by comparing mortalities from a standard concentration-mortality curve (0 UV) with the mortalities obtained after irradiation. A 5-min UV exposure period was selected (UV-A/UV-A system), because some virus activity still remained and relative comparisons could still be made for initial virus concentrations of 1,000 PIB/cup (=0.747 PIB/mm^2), 10,000 PIB/cup (=7.473 PIM/mm^2), and 100,000 PIB/cup (=74.738 PIB/mm^2). At the highest concentration (1,000,000 PIB/cup or 747.384 PIB/mm^2), enough active virus still; remained to cause >99% mortalities among *H. zea* and *S. exigua* larvae. For HzSNPV, a 5-min UV exposure reduced the "active" virus from 1,000 PIB/cup (=initial concentration) to <67 PIB/cup (=a virus concentration causing <10% mortality). As the virus concentration increased to 10,000 PIB/cup, "active" virus was reduced to ≈100 PIB/cup (=a virus concentration causing ≈20% mortality) after 5 min UV. As the initial virus concentration increased to 100,000 PIB/cup, "active" virus was reduced to ≈600 PIB/cup (=a virus concentration causing ≈50% mortality)after 5 min UV. Using activities to compare post-UV irradiation activity with activities extrapolated from a concentration-mortality curve (0 UV), it may be inferred that 94–99% of NPV was inactivated (5 min UV), regardless of virus concentration. In other words, as the virus concentration was increased, the amount of "active" virus remaining was still sufficient to cause mortality among *H. zea* larvae. As the UV period was increased, virus-caused mortality occurred only at the highest NPV concentration (=1,000,000 PIB/cup). At the end of the 240-min UV exposure period, <67 PIB/cup were still active.

For SeMNPV a 5-min UV exposure (UV-B/UV-A saystem) reduced the "active" virus from 1,000 PIB/cup to ≈300 PIB/cup (=a virus concentration causing 40% mortality). As the initial concentration increased to 10,000 PIB/cup, "active" virus was reduced to ≈1,700 PIB/cup (=a virus concentration causing ≈70% mortality) after a 5-min UV exposure. As the initial virus concentration increased to 100,000 PIB/cup, "active" virus was reduced to ≈1,000 PIB/cup (=a virus concentration causing 65% mortality). At the highest virus concentration used (=1,000,000 PIB/cup), >99% virus-caused mortality still occurred after 5- and 10-min UV exposures. After a 60-min exposure, ≈1,000 "active" PIB/cup still remained and produced 65% mortality. Further reductions in activity continued as the UV exposure period increased, and 67

"active" PIB/cup remained after a 240-min UV exposure.

Results obtained with the fluorescent tubes were repeatable from replicate to replicate and from virus to virus. These tubes are easily accessible and inexpensive and overall results (e.g., virus concentration versus time of radiation exposure) were very similar to those obtained with a Xenon solar simulator (M.S., unpublished data). Further research will more precisely pinpoint the wavelengths responsible for virus inactivation and the relationships between UV and visible radiation in virus inactivation. In addition, different radiation absorbers, reflectors, antioxidants, and radical scavengers will be tested to develop more efficacious virus formulations for insect pest control.

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